OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® FastFilter Plasmid DNA Mega Kit

D6929-00	2 preps
D6929-02	20 preps

August 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® FastFilter Plasmid DNA Mega Kit

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Introduction

The E.Z.N.A.® family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is the Omega Bio-tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E.Z.N.A.® FastFilter Plasmid DNA Mega Kit combines time-tested consistency of alkaline-SDS lysis of bacterial cells with Omega Bio-tek's innovative high efficiency DNA binding technology to recover large scale, high-quality plasmid DNA. This new method facilitates the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. This kit uses a syringe-format system that is designed to work in combination with the centrifugation steps following the alkaline lysis of bacterial cells. The Lysate Clearance Filter Syringe completely removes SDS precipitates and clears bacterial lysates in a fraction of the time required by centrifugation alone.

Yields vary according to plasmid copy number, *E. coli* strain, and conditions of growth, but 500 mL overnight culture in LB medium typically produces 2.5 mg high copy plasmid DNA. Up to 1 liter overnight culture may be processed when working with low copy number plasmids. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.

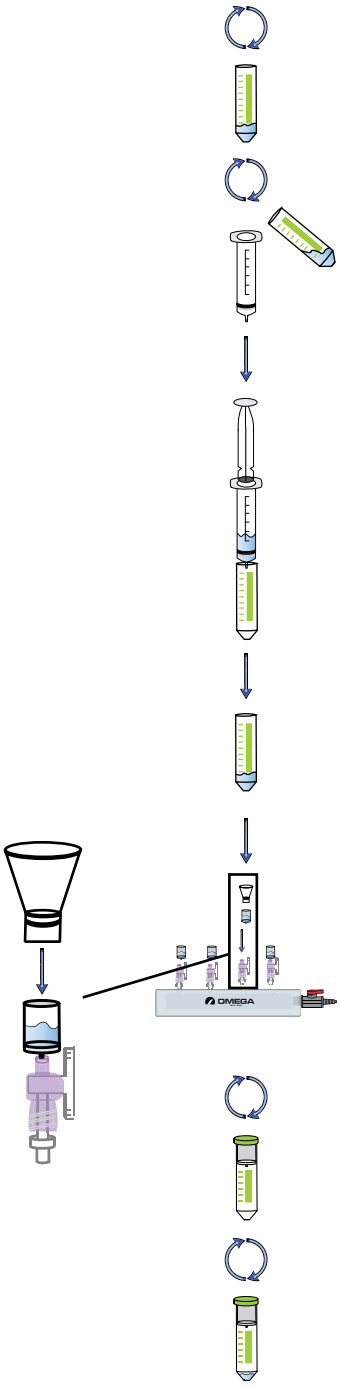
New in this Edition

- This kit now includes FastFilter Funnels to aid in sample/buffer loading of the HiBind® DNA Mega Column.

Vacuum Manifold Required

- Since large volume of buffers are used in this protocol, it is strongly recommended to use a vacuum manifold to significantly reduce the hands on time. For information on vacuum manifold settings and user instructions, please see Page 6.

Illustrated Protocol



Centrifuge cells for 10 minutes

Resuspend

Lyse

Neutralize

Spin

Transfer lysate to Lysate Clearance Filter Syringe

Insert plunger into syringe and clear lysate into a new centrifuge tube

Prepare Vacuum Manifold

Adjust binding conditions

Insert FastFilter Funnel into HiBind® DNA Mega Column

Insert funnel/column into leur connector on vacuum manifold

Pass lysate through funnel/ column

Wash 3X

Transfer column to new centrifuge tube

Centrifuge to dry

Insert column in new centrifuge tube

Centrifuge to elute

Kit Contents

Product Number	D6929-00	D6929-02
Purifications	2 preps	20 preps
HiBind® DNA Mega Columns	2	20
50 mL Collection Tubes	2	20
Lysate Clearance Filter Syringe	2	20
FastFilter Funnel	2	20
Solution I	50 mL	450 mL
Solution II	50 mL	450 mL
Neutralization Buffer	50 mL	450 mL
PFC Binding Buffer	50 mL	450 mL
PFW Wash Buffer	40 mL	450 mL
DNA Wash Buffer	25 mL	200 mL
Elution Buffer	15 mL	125 mL
RNase A	450 µL	4 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® FastFilter Plasmid DNA Mega Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. RNase A should be stored at 2-8°C. Solution I/RNaseA should be stored at 2-8°C after being combined (see Page 5). All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in Solution II and PFC Binding Buffer. Dissolve such deposits by warming the solution at 50°C and gently shaking.

Preparing Reagents

- Add vial of RNase A to the bottle of Solution I provided and store at 2-8˚C.

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D6929-00	100 mL
D6929-02	800 mL

Recommended Settings

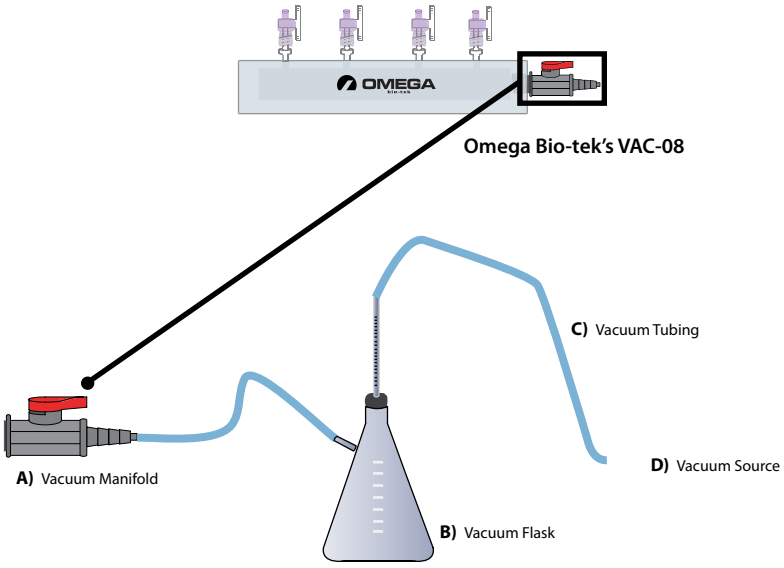
The following is required for use with the Vacuum/Spin Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an end A negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α™ , DH1, and C600. These host strains yield high-quality DNA with E.Z.N.A.® FastFilter Plasmid DNA Mega Kit Protocols. XL1-Blue, although a slower growing strain is also recommended due to its yield of high-quality DNA.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activity when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101). One may reduce the amount of culture volume or double the volumes of Solution I, Solution II, and N3 Buffer, if problems are encountered with strains such as TG1 and Top10F.

Inoculation

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 at 37°C with vigorous shaking (~300 rpm; shaking incubator).

Note: Aeration is very important. The culture volume should not exceed 1/4 the volume of the container.

Culture Media

E.Z.N.A.® FastFilter Plasmid DNA Mega Kits are specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB(Terrific Broth) or 2 x YT lead to high cell densities that can overload the purification system, and therefore are not recommended. If rich media has to be used, growth times have to be optimized, and the recommended culture volumes must be reduced to match the capacity of the HiBind® DNA Mega Column.

Note: As culture ages, DNA yield may begin to decrease due to cell death and lysis within the culture.

Culture Volume and Cell Density

Do Not Exceed Maximum Recommended Culture Volumes

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD_{600} is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD_{600} of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

E.Z.N.A. FastFilter Plasmid DNA Mega Kit Protocol

This protocol is designed to isolate 2.5 mg high copy number plasmid DNA or 200-500 µg low copy number plasmid DNA from 500 mL overnight cultures.

Materials and Equipment to be Supplied by User:

- 100% Ethanol
- Centrifuge with swing bucket rotor capable of 5,000 x *g* with adapters for 50 mL centrifuge tubes and 125 mL or 250 mL centrifuge bottles
- Vacuum pump capable of generating -200 to -600 mbar
- Vacuum Manifold (Cat No. VAC-08)
- 50 mL centrifuge tubes
- 125 or 250 mL centrifuge bottles
- Centrifugation tubes (i.e Nalgene 3120)
- Vortexer
- Ice bucket

Before Starting:

- Chill Neutralization Buffer on ice
- Prepare DNA Wash Buffer and Solution I according to the "Preparing Reagents" section on Page 5

1. Inoculate 500 mL LB/ampicillin (50 µg/mL) medium placed in a 2-5 liter culture flask with *E. coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 hours. It is strongly recommended that an end A negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

   Note: Optimal growth conditions of bacteria is vital of obtaining maximal plasmid DNA yields. The best conditions are achieved by picking a single colony from a freshly transformed or freshly grown plate to inoculate a 2-5 mL starter culture containing the appropriate antibiotic. Incubate for ~8 hours at 37°C with vigorous shaking (~300 rpm). Using a flask or vessel with a volume of a least 3-4 times the volume of the culture, dilute the starter culture 1/500 to 1/1000 times into warm culture media containing antibiotics. Grow at 37°C for 12-16 hr with vigorous shaking (~300 rpm).

   Following overnight bacterial growth, an OD_{600} of 1.5~2.0 indicates a well-grown culture. For the best result determination of OD_{600} for each culture is recommended. It is important to dilute the bacterial culture (10 to 20 fold) to enable photometric measurement in the linear range between 0.1 and 0.5 OD_{600}. We recommend a bacterial density of between 2.0 and 3.0 at OD_{600}. When using nutrient-rich media, care should be taken ensure that the cell density does not exceed an OD_{600} of 3.0. If using a frozen glycerol stock as the inoculum, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. Then pick a single colony and inoculate the 2-5 mL starter culture as described above.

2. Pellet the bacteria in an appropriate vessel by centrifugation at 5,000 × *g* for 10 minutes at room temperature.

3. Decant or aspirate the media and discard. To ensure that all traces of media are removed, use a clean paper towel to blot excess liquid from the wall of the vessel.

4. Add 20 mL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to Solution I before use. Please see the instructions in the "Preparing Reagents" section on Page 5.

5. Add 20 mL Solution II. Invert and rotate the tube gently 10 times to obtain a cleared lysate. Let sit at room temperature for 3 minutes.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Add 20 mL cold Neutralization Buffer. Gently invert 10 times or until a flocculent white precipitate forms. This may require a 2 minute incubation at room temperature with occasional mixing.

   Note: The solution must be mixed thoroughly. This is vital for obtaining good yields. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Centrifuge at 4,000 x *g* for 5 minutes. The lysate will not completely clear until after Step 8.

   Note: Increasing centrifugation speed is helpful to completely remove the precipitated bacterial cell debris. After centrifugation, a tightly packed pellet indicates efficient lysis.

8. Transfer the lysate into the barrel of the Lysate Clearance Filter Syringe. Allow the lysate to sit for 2 minutes.

9. Hold the Lysate Clearance Syringe barrel over a 125 mL or 250 mL centrifuge tube (not provided) and remove the end cap from the syringe tip.

10. Gently insert the plunger into the barrel to expel the cleared lysate into the 125 mL or 250 mL centrifuge tube.

    Note: Some of the lysate may remain in the flocculent precipitate. DO NOT force this residual lysate through the filter.

11. Measure the volume of the supernatant.

12. Add 1/3 volume PFC Binding Buffer. Vortex to mix thoroughly.

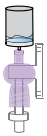
13. Prepare the vacuum manifold by following the manufacturer's instructions.

14. Insert the FastFilter Funnel into the HiBind® DNA Mega Column. Refer to the Illustrated Protocol on Page 3 for details.

15. Connect the HiBind® DNA Mega Column/Funnel to the vacuum manifold. Refer to the Illustrated Vacuum Setup on Page 6 for details.

16. Transfer all the cleared lysate from Step 12 by CAREFULLY decanting it into the HiBind® DNA Mega Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mega Column.

    Note: Do not allow the HiBind® DNA Mega Column to become empty until you have transferred all of the lysate from Step 12.



Leaving ~1 mL solution in the column after Step 16 reduces foaming and clogging which decreases total processing time and improves efficiency.

17. Turn on the vacuum source to draw the lysate through the column.

18. Turn off the vacuum.

19. Repeat Steps 16-18 until all of the cleared lysate has been transferred to the HiBind® DNA Mega Column.

20. Add 20 mL PFW Wash Buffer.

21. Turn on the vacuum source to draw the buffer through the column.

22. Turn off the vacuum.

23. Add 20 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 5.

24. Turn on the vacuum source to draw the buffer through the column.

25. Turn off the vacuum.

26. Repeat Steps 23-25 for a second DNA Wash Buffer wash step.

27. Remove the HiBind® DNA Mega Column/Funnel from the vacuum manifold. Discard the FastFilter Funnel.

28. Transfer the HiBind® DNA Mega Column to a 50 mL Collection Tube (provided).

29. Centrifuge the empty HiBind® DNA Mega Column at 5,000 x *g* for 10 minutes to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mega Column matrix before elution. Residual ethanol may interfere with downstream applications.

30. Transfer the HiBind® DNA Mega Column to a nuclease-free 50 mL centrifuge tube (not provided).

31. Add 1.5-3 mL Elution Buffer directly to the center of the column matrix.

32. Let it sit at room temperature for 5 minutes.

33. Centrifuge at 5,000 x *g* for 5 minutes.

    Note: This represents approximately 65-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration.

34. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, **at 1-800-832-8896.**

Possible Problems and Suggestions

Low DNA Yields	
Poor cell lysis	• Reduce the initial volume of culture or increase the lysis time while monitoring the lysis visually. • Cells may not have been dispersed adequately prior to the addition of Solution II. Make sure to vortex cell suspension to completely disperse bacterial cells. • Solution II, if not tightly closed, may need to be replaced.
Bacterial culture is overgrown or not fresh	• Do not incubate cultures for more than 24 hr at 37°C. • Storage of cultures for extended periods prior to plasmid isolation is detrimental.
Low elution efficiency	If using endotoxin-free water for elution, adjust the pH of the water to pH 8.0
Mega Columns were spun in a fixed angle rotor or with insufficient g-force	The HiBind® DNA Mega Columns must be spun in a centrifuge with a swing bucket rotor at 5,000 x *g* for liquids to pass through efficiently.
Alkaline lysis was prolonged	Reduce the lysis time (Solution II) to 3 minutes or until the suspended cells form a clear viscous solution.
Too many or too few cells were used	Confirm the cell density by measuring OD. To calculate the volume of culture to use, take the desired cell mass and divide by the absorbance of the overnight culture at 600 nm.
DNA Wash Buffer not diluted with ethanol	Prepare DNA Wash Buffer as instructed on Page 5.
High molecular weight DNA contamination of product.	
Over mixing of cell lysate upon addition of Solution II	Do not vortex or mix aggressively after adding Solution II.
Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.

Plasmid DNA floats out of well while loading agarose gel	
Ethanol has not completely been removed from column following wash steps	Centrifuge column as instructed to dry the column before elution. Incubate columns for 10 minutes at 65°C to completely dry membrane after centrifugation step for drying
Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A_{260} /A_{280} ratio is high or low).	
DNA Wash Buffer was diluted with ethanol containing impurities	Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the Mega Column after washing and contribute to the absorbance in the final product.
Plasmid DNA was contaminated with RNA; RNase A treatment is insufficient	Confirm that the RNase A was added to Solution I prior to first use. RNase A may degrade due to high temperatures (> 65ºC) or prolonged storage (> 6 months at room temperature).
Background reading is high due to silica fines	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
Purification is incomplete due to column overloading	Reduce the initial volume of culture.
Plasmid DNA is contaminated with chromosomal DNA	Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during the lysis reaction or Neutralization Buffer step.
4,000 x *g* centrifuge not available	
4,000 x *g* centrifuge not available	For centrifuges only capable of 2,000-4,000 x *g*, increase all centrifugation times by 2 minutes except for the drying of the column. Increase drying by 5 minutes. It may be necessary to incubate the empty column after the drying step at 65°C for 10 minutes to completely dry the column ***A Swing Bucket Centrifuge is Required.***

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Vacuum Manifold	VAC-08
Solution I (250 mL)	PS001
Solution II (250 mL)	PS002
Neutralization Buffer (250 mL)	PS004
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (100 mL)	PS010
RNase A (5 mL)	AC117